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University of California
School of Public Health
Berkeley, California 94720

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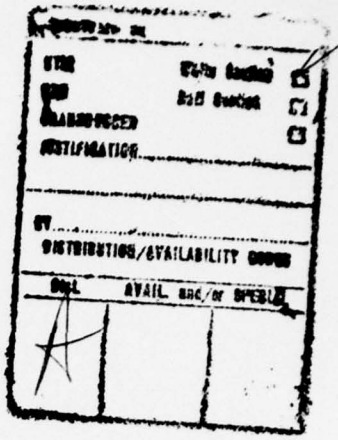

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SUMMARY

During this past year we completed the testing, by passive hemagglutination inhibition test (PHI), of Culex tarsalis collected during the period 1960-65 in Kern County, California and previously shown to have fed on passeriform birds by the precipitin test. It was shown that these mosquitoes fed principally on House finches and House sparrows, the most common passeriform birds found in the collection areas. These data demonstrated the usefulness of the PHI test for the identification of arthropod blood meals from closely related species.

Several species of blood engorged mosquitoes from Malaya were tested. Only one species, Culex cinctellus, was collected in any significant number and they were shown to have fed on both warm and cold-blooded animals.

Collaborative studies with two units concerned with the Epidemiology of Chagas' disease in Mexico and Panama submitted blood engorged triatomid bugs to us for testing. In Mexico Triatoma barberi were collected. They were shown to have fed primarily on man, cricetid and murid rodents. A few fed on domestic birds. Only one blood meal from Rhodnius pallescens submitted from Panama was identified.

In the developmental work, we demonstrated that sheep red blood cells treated with pyruvic aldehyde, conjugated with purified antibody and stored at freezer temperatures (-20°C, -70°C) up to 8 weeks were usable in the reversed passive hemagglutination test. The sensitivity of these cells increased during storage.

INTRODUCTION

Since the start of this research program in July 1970, we have been concerned with two primary goals. The first has been to improve the immunologic tools available to the Medical Entomologist and Epidemiologist so as to enable them to accurately determine the host ranges of arthropod vectors of human and animal diseases. The second goal was to test arthropod blood meals of known vectors from several areas of the world. This required the establishment of numerous collaborative studies with laboratories and field units found primarily in tropical and sub-tropical areas. In addition, this laboratory continued its long collaborative study with University of California Arbovirus Unit during this peiod.

Results relating to these efforts were presented in 5 previous annual progress reports. This final report will include more detailed data acquired since the last annual report and will reflect the reduced level of funding received during the current fiscal year. In addition, a summary of the prior five years research is included.

PART I: HOST PREFERENCES

A. Identifications of Feedings on Passeriform birds by Culex tarsalis collected in Kern County 1960-65.

An intensive effort was made from 1960-65 to collect blood engorged Culex tarsalis in Kern County, California and when these blood meals were identified by the precipitin test, 46.4 percent were from passeriform birds[1]. This group of birds represented the predominant avian group in Kern County and were the most important source of vector infection with western equine encephalomyelitis, St. Louis encephalitis and Turlock viruses[2-4]. Limitations in the sensitivity and specificity of the precipitin test did not allow us to identify the species of passeriform birds the C. tarsalis had fed upon.

The adaptation of the passive hemagglutination test (PHI) to mosquito blood meal identification with its inherent sensitivity and the purification of antibody by affinity chromatography with its resultant high degree of specificity permitted us to determine more exactly the species of origin of blood meals[5]. This sensitive and specific adaption of the PHI test was developed during the period of these contracts.

In this study, 4,209 specimens collected during the 1960-65 period were tested. Approximately 50 percent of this number were tested in prior contract years and reported in other annual reports. These data, as well as those mosquitoes tested this past year are reported (Table 1).

Of the 4,209 specimens tested and previously shown to have fed on passeriform birds by the preciptitin test, the majority either fed on House finches (30.4%) or House sparrows (31.8%), (Table 1). Blackbirds were fed on significantly less (10.6%) than were the White-crowned sparrows (1.9%). Over 25% of the blood meals remained unidentified.

Antisera that were specific for 3 species of Passeriformes and family specific for the Icteridae were used in this study. The direction and limitations in tests were dictated by the following criteria: the relative abundance of the several passeriform species in Kern County, the suspected role of the species in the epidemiology of arboviruses, the availability of a sufficient quantity of blood serum to serve as the antigen in preparation of the reagents, and the amount of blood meals from individual mosquitoes that was available following earlier tests.

Definitive data were achieved by using the PHI method, but as yet this test system cannot supplant the precipitin test as a screening or easily established test system. The reagents necessary for the precipitin test can be easily produced, the test is rapid and the results obtained will place a blood source directly into a major animal grouping. This study, however, confirms the usefulness of the PHI technique for the identification of blood meals from closely related species and expands the study potential in areas of mosquitoe host preferences.

B. Identification of feeding from miscellaneous mosquitoes collected in Malaya.

Mosquitoes representing 13 species were collected in the Gunong Besout Forest Preserve, Malaya, and submitted to our laboratory for blood meal identifications. These mosquitoes were collected by the University of California International Center for Medical Research, Arbovirus Research Unit. Only one species, Culex cinctellus, were collected in any significant number. Of the 131 mosquitoes submitted for testing, only 42 blood meals were identified, the others either had too little or no blood. Those blood meals identified indicated that this mosquito fed equally well on both warm and cold-blooded animals (Table 2).

The number of identified blood meals from all of the remaining mosquitoes tested was too small to determine any feeding trends (Table 2). No blood meals from five species of mosquitoes were identified.

C. Feeding patterns of Triatoma barberi collected in Mexico.

In a collaborative study of Chagas' Disease Epidemiology being carried out by a group consisting of persons from both the Berkeley and San Francisco Campuses of the University of California, we have tested 219 Triatoma barberi collected in Magadelena Apazo, Oaxaco, Mexico. This Triatomid bug is the principal vector of Chagas' disease in this area.

From the 219 bugs tested by the precipitin test, 247 blood meals were identified. Twenty of the Triatomids contained little if any blood and no blood meals were identified from these specimens. Almost 25 percent of the Triatomids contained more than one identified blood meal. This is a significant number of multiple feeding insects when compared with mosquitoes which, in our testing program, have averaged below 1 percent. The majority of the blood meals were taken from various mammals. Man, Cricetid and Murid rodents served as hosts for approximately 60 percent of the Triatomids while the remaining blood meals were from domestic mammals and birds (Table 3).

This study is continuing and should provide important information on the epidemiology of Chagas' Disease.

D. Testing of the Triatomine Bug Rhodnius pallescens collected in Panama.

Because of the termination of the blood meal identification program, this study was phased out early in the year. Only 20 Rhodnius pallescens collected in Panama were submitted for testing. Of this number only 1 blood meal was identified and this specimen fed on a mammal. Further collaboration should have increased both the number and better specimens.

This study, like the one previously reported, is part of a program concerned with establishing the epidemiology of Chagas' disease.

PART II: DEVELOPMENTAL STUDIES

In last year's annual summary we reported the need for the long term storage of sheep red blood cells (SRBC) conjugated with various antigens or antibodies so as to eliminate the necessity of frequent conjugation as well as to save precious reagents. A preliminary study was detailed and the results stated in that report. We have confirmed our preliminary data and have extended it this past year.

In this study SRBC were stabilized with 1.2% aqueous solution of pyruvic aldehyde. Antibodies were produced in the chicken and rabbit to bovine serum albumin (BSA) and deer serum albumin (DSA) and subsequently purified by affinity chromatography. The purified antibody was added to the pyruvic aldehyde treated cells in various µg quantities.

The cells were divided up into several lots and paired lots were separated and stored at various temperatures for different time periods. One of the two in each pair was diluted in phosphate buffered saline (PBS) and the other in a diluent of PBS to which 0.1% gelatin was added. Aliquots were stored at 4°C, -20°C and -70°C up to 8 weeks. Those that were held at freezer temperatures were quick frozen in dry ice and acetone. All assays were made in microtiter V-plates and by use of the reverse passive hemagglutination method (RPHA).

The results obtained indicated that those cells stored at -20°C and -70°C remained stable over the length of the experiment. In fact, sensitivity increased during the storage period (Table 4). Cells stored at 4°C were not satisfactory after about two weeks of storage. The most satisfactory results in terms of sensitivity were with those cells stored in PBS containing gelatin (Table 5). Specificity was not lost during the storage period as mosquito blood meals obtained from several mammalian and bird sources were tested with those cells and only the homologous blood meals reacted to any significant degree.

The advantages of the RPHA technique using the cells described here overcomes two deficiencies of the PHA technique adapted to the testing of mosquito blood meals[5]. Tests can be completed in one day by the direct incubation of the antibody-coated cells with the blood meal. In addition they can be stored indefinitely at freezer temperatures with an apparent increase in sensitivity. Tha ability to store cells for long periods provide two important solutions to existent problems. It provides stable reagents during the course of a long-term study. In addition, it would make available key reagents, of known quality, that would be available to investigators that are inexperienced in producing and evaluating such reagents.

PART III: SUMMARY 1970-75

A. Host preferences

1. Panama

a. Mosquitoes

The host feeding patterns of 6 Culex (Melanoconion) mosquitoes and 1 Culex (Aedinus) mosquitoes species collected and submitted to the Berkeley laboratory were tested by the precipitin test. The mosquitoes included: Culex (M.) aikenii, C. (M.) egcymon C. (M.) tecmarsis, C. (M.) dunni, C. (M.) elevator, C. (M.) epanastasis and C. (A.) amazonensis. Approximately 3,000 blood meals were identified. Culex aikenii showed considerable versatility in feeding pattern. It fed equally well on birds and mammals. Significant numbers fed on echimyid rodents and ciconiiform birds. Cold-blooded animals, principally lizards also served as hosts.

Culex egcymon, C. tecmarsis, C. dunni and C. elevator fed predominantly on lizards. Culex dunni also fed on birds and mammals.

Culex epanastasis fed readily on both warm and cold-blooded animals while C. amazonenis showed almost a complete preference for mammals(6). (See also 1972-73 annual report).

b. Triatomines

A preliminary investigation was commenced to study the host preferences of Triatomines was commenced. Data secured during the course of this study were mostly negative, because of the unsatisfactory material received.

2. Butte and Glenn Counties, California

Collections of blood engorged mosquitoes were made in Butte and Glenn Counties, California by the University of California arbovirus unit, during the period extending from 1969 through 1973. Seventeen species were collected and over 12,000 mosquitoes blood meals were identified (See 1974-75 annual report).

a. Culex mosquitoes

Almost 4800 Culex tarsalis blood meals were identified during this period. It demonstrated a catholic feeding pattern with a preference for birds. As has been demonstrated previously for this mosquito, it was shown that the feeding shifted away from birds to mammals commencing in the mid-summer.

Other Culex species tested included: C. peus, C. peus-thriambus, C. erythrothorax and C. pipiens. Culex erythrothorax fed on mammals; the other species principally on birds.

b. Anopheles mosquitoes

The species of Anopheles mosquitoes collected during this study included: A. freeborni, A. franciscanus and A. punctipennis. Over 5500 A. freeborni blood meals were identified. This species fed predominantly on the ruminant group. Almost 25 percent fed on leporids. Basically the same patterns were observed for A. franciscanus and A. punctipennis.

c. Aedes mosquitoes

Four species of Aedes mosquitoes were collected and tested. The species collected in the largest number was Aedes melanimon (716). This mosquito fed almost entirely on mammals principally leporids. Lesser numbers of A. nigromaculis were tested and these three species preferred mammals.

3. Influence of mosquito population densities on the host feeding selection.

Stable traps, each baited with a jackrabbit and either a chicken or pheasant, collected more than 21,000 mosquitoes in the Sacramento Valley, California in 1972 and 1973. The primary concern of this study was the effect of mosquito densities on the feeding behavior of C. tarsalis. Feeding success was usually less and feeding rates on the jackrabbit were greater when large numbers of mosquitoes were collected or when a bird was exposed that was less receptive to mosquito feeding. Greater feeding rates on the jackrabbit apparently resulted from decreased feeding on the bird and a diversion of mosquitoes to the jackrabbit.

B. Developmental studies

1. Passive Hemmagglutination test

During this period we have adopted in the passive hemagglutination technique for use in the identification of arthropod blood meals. This method is a sensitive and specific technique and, in many cases, we are now able to identify blood meals to the species level[5,7]. The antisera for the tests are prepared in chickens using chemically pruified antigens. The antibody obtained is purified by immunoadsorption. Several thousand blood meals previously identified by the precipitin test to taxonomic orders or families were identified to the genus or species level by this test[7].

A modification of this test was also adapted to blood meal identification. It requires the direct reaction of the erythrocyte to which antibody has been conjugated and the blood meal. This direct reaction of the two reagents greatly reduces the time and the amount of reagents needed to run the test. It appears to be a usable and practical test. However, we have not used it extensively for arthropod blood meal identification. The test is called the reverse passive hemagglutination test.

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TABLE 1. Definitive identification by the passive hemagglutination inhibition test of blood meals from Culex tarsalis collected 1960-65 in Kern County, Califronia and previously shown to have fed on passeriform birds by the precipitin test.

	Total Feedings	House Finch	House Sparrow	Blackbird	White-crowned Sparrow	Negative
Number	4220[1]	1284	1344	447	78	1067
Percent		30.4	31.8	10.6	1.9	25.3

[1]Includes 11 double feedings.

TABLE 2. Feedings of various mosquito species collected in the Gunong Besout Forest Preserve, Malaya 1974-75

Mosquito Species	Host														
			Mammal					Bird				Reptile	Amphibian		
	Number Tested	Negative	Horse	Unknown Rodents	Monkey	Negative	Subtotal	Chicken	Passerine	Negative	Subtotal	Unidentified	Frog	Total	
Culex cinctellus	131	82	1	14		1	16	12	4	10	26	6	1	131	
Culex pseudovishnui	2			1						1				2	
Culex (lopho)sp	14	3		2			2	1			1	5	3	14	
Culex nigropunctatus	1			1										1	
Aedes Neomacleaya sp.	5	4						1			1			5	
Aedes (Finlaya) albocinctus	1			1			1							1	
Anopheles umbrosus	1				1		1							1	
Culiciomyia sp.	3	1		1	1		2							3	
Total	158	90	1	20	2		22	14	4	11	28	11	4	158	

TABLE 3. Feeding patterns of Triatoma barberi collected in Magdalena Apazco, Oaxaco, Mexico

	Host														
	Mammal									Bird			Amphibian	Total Reactions	Non-Reactor
	Man	Bovine	Dog	Cat	Horse	Murid Rodent	Cricetid Rodent	Heteromyid Rodent	Negative	Turkey	Chicken	Negative	Unidentified		
Number	47[1]	9[2]	14[3]	14[4]	2[5]	45[6]	46[7]	1	20	23[8]	17[9]	4	5	247	20
Percent	19.0	3.7	5.7	5.7	0.8	18.2	18.6	0.4	8.1	9.3	6.9	1.6	2.0	100.0	

1. Includes 10 double feedings
2. Includes 6 double feedings
3. Includes 5 double feedings
4. Includes 6 double feedings
5. Includes 1 double feeding
6. Includes 9 double feedings
7. Includes 4 double feedings
8. Includes 4 double feedings
9. Includes 4 double feedings

TABLE 4. Sensitivity of purified chicken anti-BSA conjugated to pyruvic aldehyde treated cells and stored at various temperatures in two diluents for different time periods.[1]

Day	Temperature					
	4°C	-20°C	-70°C	4°C	-20°C	-70°C
		Stored in PBS			Stored in Gelatin Diluent	
1	39.0[2,3]	78.0	2.4	4.8	4.8	4.8
3	156.0	9.7	9.7	9.7	39.0	78.0
7	156.0	78.9	3.9	9.7	39.0	78.0
14	1250.0	39.0	9.7	78.0	39.0	19.0
42	—[4]	4.8	4.8	—[4]	625.0	312.0
56	—	4.8	1.2	—	78.0	19.0

1. 75 µg of antibody/0.1 ml stabilized SRBC.

2. Initial dilution of antigen was 50 µg/ml.

3. End point in nanograms.

4. Cells non-specifically agglutinated.

TABLE 5. Sensitivity of purified rabbit anti-BSA and chicken anti-DSA conjugated to pyruvic aldehyde cells and stored at -20°C and -70°C when tested against various dilutions of mosquito blood meals.

TEMPERATURE				
	-20°C	-70°C	-20°C	-70°C
	Anti-BSA[1]		Anti-DSA[2]	
DAY			DAY	
0	6.56×10^{-4}[3,4]		0	3.28×10^{-4}
4	4.08×10^{-3}	1.23×10^{-4}	5	4.08×10^{-3}* 1.84×10^{-4}
8	3.06×10^{-3}	1.64×10^{-4}	7	3.69×10^{-4} 4.92×10^{-4}
11	8.16×10^{-3}	4.59×10^{-3}	10	1.64×10^{-4} 9.22×10^{-3}
14	2.04×10^{-3}	1.64×10^{-4}	14	NT 3.56×10^{-4}
21	6.56×10^{-4}[5]	3.06×10^{-3}	21	2.04×10^{-3} 4.08×10^{-3}
28	NT	NT	28	4.08×10^{-3} 1.64×10^{-4}[5]
35	3.28×10^{-4}[5]	6.56×10^{-4}	35	1.31×10^{-5} 3.69×10^{-4}
46	$>2.46 \times 10^{-5}$[6]	$>2.62 \times 10^{-5}$[6]	49	3.28×10^{-4} $>2.62 \times 10^{-5}$[6]
56	1.31×10^{-5}	2.62×10^{-5}	56	7.37×10^{-4} 2.62×10^{-5}

1. 200 µg of antibody required per 0.1 ml stabilized cells for optimal results.
2. 25 µg of antibody required per 0.1 ml stabilized cells for optimal results.
3. Initial dilution of blood meal assumed to be around 1:1,000.
4. Average of samples.
5. Only one sample.
6. End point not determined.

Publications and Manuscripts in Preparation

Tempelis, C.H. and Rodrick, M.L. Passive hemagglutination inhibition technique for the identification of arthropod blood meals. Am. J. Trop. Med. Hyg. 21:238-245, 1972.

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Tempelis, C.H. and Aguayo, E. The effect of storage on pyruvic aldehyde treated erythrocytes conjugated with serum protein antigen. In preparation.

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Triatoma barberi
Rhodnius pallescens

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

For Abstract see reverse page (block 20)

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During this past year we completed the testing, by passive hemagglutination inhibition tests (PHI), of Culex tarsalis collected during the period 1960-65 in Kern County, California and previously shown to have fed on passeriform birds by the precipitin test. It was shown that these mosquitoes fed principally on House finches and House sparrows, the most common passeriform birds found in the collection areas. These data demonstrated the usefulness of the PHI test for the identification of arthropod blood meals from closedly related species.

Several species of blood engorged mosquitoes from Malaya were tested.

Collaborative studies with two units concerned with the Epidemiology of Chagas' disease in Mexico and Panama submitted blood engorged triatomid bugs to us for testing. In Mexico Tratoma barberi were collected. They were shown to have fed primarily on man, cricetid and murid rodents. A few fed on domestic birds.

In the developmental work, we demonstrated that sheep red blood cells treated with pyruvic aldehyde, conjugated with purified antibody and stored at freezer temperatures (-20°C, -70°C) up to 8 weeks were usable in the reversed passive hemagglutination test. The sensitivity of these cells increased during storage.